Review

Leitmotifs in the biochemistry of LTP induction: amplification, integration and coordination

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Abstract

Hippocampal long-term potentiation (LTP) is a robust and long-lasting form of synaptic plasticity that is the leading candidate for a cellular mechanism contributing to mammalian learning and memory. Investigations over the past decade have revealed that the biochemistry of LTP induction involves mechanisms of great subtlety and complexity. This review highlights themes that have emerged as a result of our increased knowledge of the signal transduction pathways involved in the induction of NMDA receptor-dependent LTP in area CA1 of the hippocampus. Among these themes are signal amplification, signal integration and signal coordination. Here we use these themes as an organizing context for reviewing the profusion of signaling mechanisms involved in the induction of LTP.

Keywords: CA1, excitability, kinase, long-term potentiation, memory, review.


Tremendous progress has been made enhancing our knowledge of the various signal transduction cascades necessary for the induction and maintenance of NMDA receptor-dependent long-term potentiation (LTP) in hippocampal area CA1. There is a bewildering array of detail now available concerning the second messengers, signaling molecules and protein kinases whose activities are necessary for the induction of LTP. Moreover, many of these signaling cascades interact with each other, yielding a biochemistry of LTP induction that can be described as baroque in its complexity.

However, as the details concerning the identities of the signaling molecules in LTP induction have become known, it seems to us that certain unifying and harmonious themes have begun to emerge. The goal of this review is to highlight these themes in order to provide a framework for organizing recent findings and for incorporating new findings as they emerge. We will not be able to review in detail all the relevant primary literature due to space constraints. Thus, we will limit ourselves to the citation of specific examples of the properties of the signaling molecules involved in LTP induction, in the context of the attributes that they have in common with other molecular participants in LTP.

What are the leitmotifs1 for the biochemistry of LTP induction? In our minds three unifying principles have recently emerged: signal amplification, signal integration, and signal coordination (Table 1). Signal amplification is perhaps the most striking theme that emerges from an evaluation of the known biochemical mechanisms contributing to LTP induction. We will present six examples from the literature for this theme; doubtless there are many others. Signal integration is another robust theme to emerge from studies of signal transduction in LTP. We will divide this theme in three subcategories: temporal integration, wherein repeated presentation of a stimulus elicits specific effects; functional integration, which arises from multiple signal transduction cascades converging on a final common effector; and spatial integration, wherein signals originating in different parts of the cell converge to elicit in combination unique responses. Signal coordination is the final theme we will address. This theme highlights the importance of a given

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1 leitmotif n. Music A recurring theme used for a certain person, event, or idea throughout an opera, etc. (Funk & Wagnalls Standard Encyclopedic Dictionary).

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Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; β-AR, β-adrenergic receptor; BDNF, brain-derived neurotrophic factor; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; CREB, Ca²⁺/cAMP response element binding protein; DA, dopamine; EPSP, excitatory postsynaptic potential; erk MAPK, extracellular-regulated mitogen-activated protein kinase; LTP, long-term potentiation; MEK, MAP/ERK kinase; NO, nitric oxide; PKA, cAMP-dependent protein kinase; PKC, Ca²⁺/phospholipid-dependent protein kinase.
signal transduction cascade eliciting a coordinated cellular response by its activity on multiple downstream effectors.

**Definitions and established themes in LTP biochemistry and physiology**

Before proceeding to our exploration of the leitmotifs of LTP biochemistry, it is necessary to highlight the definition of three terms commonly used in discussing LTP: induction, maintenance and expression. We know that two types of biochemical mechanisms contribute to what is observed physiologically as long-term potentiation: transient and persistent biochemical phenomena. Transient biochemical phenomena, such as elevation of second messenger levels and the resulting protein kinase activation, trigger the induction of LTP. These transient events last on the order of about 10 min after LTP-inducing stimulation. In contrast, the maintenance of LTP is subserved by persisting biochemical phenomena. Contemporary models of LTP divide LTP maintenance into two phases: early LTP (E-LTP), which is subserved by persistent kinase activation, and late LTP (L-LTP), which is characterized by a dependence on protein synthesis and altered gene expression. E-LTP maintenance is executed by two enzyme species that are converted to autonomously active (i.e. second messenger independent) forms during LTP induction. In contrast, the maintenance of LTP is subserved by persisting biochemical phenomena. Contemporary models of LTP divide LTP maintenance into two phases: early LTP (E-LTP), which is subserved by persistent kinase activation, and late LTP (L-LTP), which is characterized by a dependence on protein synthesis and altered gene expression. E-LTP maintenance is executed by two enzyme species that are converted to autonomously active (i.e. second messenger independent) forms during LTP induction. Autonomously active Ca\(^{2+}\)/phospholipid-dependent protein kinase (PKC) and Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) maintain E-LTP by virtue of their long-lasting activation. The physiologic expression of LTP (that component maintained by kinase activity) is a result of CaMKII and PKC phosphorylation of substrate proteins such as ion channels and neurotransmitter receptors, whose altered properties result in the observed altered physiologic response. These transient and persistent biochemical events manifest the physiologic output termed LTP and represent the molecular transition from LTP as a biochemical phenomenon to LTP as a physiologic phenomenon.

In the context of distinguishing between LTP induction and maintenance, two themes in LTP biochemistry are already well established in the LTP literature (also listed in Table 1). First is the clear importance of persisting biochemical signals in LTP. As a persistent physiologic outcome, LTP must therefore be subserved by persisting biochemical signals in the cell. A second theme is the prominent role of protein kinases in the induction and maintenance of LTP. Protein kinases are particularly well suited for their role in the induction of LTP because they elicit rapid post-translational modifications in a tightly regulated fashion. Moreover, in the case of CaMKII and PKC, these enzymes have the capacity to be rapidly converted to a persistently activated autonomous enzyme in response to a transient second messenger signal. In the context of LTP induction as a physiologic phenomenon, a final theme already noted in the literature is the distinction between molecules that modulate the induction of LTP and those whose actions are mandatory for the induction of LTP (Table 1). The prototype modulator of LTP induction is nitric oxide (NO). This molecule is necessary for LTP induction under conditions of low intensity stimulation or at low temperatures (Gribkoff and Lum-Ragan 1992; Chetkovich et al. 1993; Haley et al. 1993; Williams et al. 1993). However, a lack of NO can be readily overcome by increasing the robustness of the physiologic LTP-inducing protocol, for example by increasing stimulus intensities or performing experiments at above room temperature.

A variation on this idea has probably not received the attention it deserves; a given molecule may serve to both modulate the induction of one phase of LTP while being requisite for the induction of another phase. For example,
several investigators have observed that PKA inhibitors or mitogen-activated protein/extracellular signal-regulated kinase (MAP/ERK kinase, MEK) inhibitors attenuate E-LTP and block L-LTP expression. Thus, the cAMP cascade and the extracellular signal-regulated kinase mitogen-activated protein kinase (erk MAPK) cascade likely modulate the induction of E-LTP but are required for the induction of late LTP (L-LTP). It will be interesting to determine if other signal transduction cascades have similar roles. Particularly interesting is the possibility that a molecule that is necessary for inducing E-LTP is a modulator for (or superfluous to) the induction of L-LTP.

**Subtypes of LTP in area CA1 of the hippocampus**

Prior physiologic studies have demonstrated that LTP in area CA1 can be broadly divided into two subtypes — induction through NMDA receptor-dependent and NMDA receptor-independent means. NMDA receptor independent LTP in area CA1 can be induced using tetraethylammonium ion application, or more commonly by using 200 Hz tetanic stimulation (Grover and Teyler 1990; Powell et al. 1994; Kanterewicz et al. 2000). As described above, the NMDA receptor-dependent form of LTP in area CA1 can be further divided into two subtypes; a transient form (E-LTP, typically lasting 1–2 h) and a long-lasting form (L-LTP, lasting at least 5 h or more).

Recent advances have provided us a much more detailed understanding of the signal transduction mechanisms operating to elicit NMDA receptor-dependent LTP in area CA1, and this will be the focus of our review. As mentioned above, one theme that has already become clear is that protein kinases play a critical role in LTP. Four protein kinases have achieved particular prominence as players in LTP induction and maintenance: PKC, PKA, CaMKII and erk MAPK. Recent biochemical studies have given us substantial insight into the time courses and mechanisms of activation of most of these kinases, and pharmacologic and genetic studies have made clear the necessity for their activation in LTP. The primary literature implicating these kinases in LTP has been reviewed recently (Sweatt 1999), and we will therefore focus on the recurring motifs that have emerged as the regulatory mechanisms of protein kinases and the identity of their downstream effectors in LTP have been elucidated.

As a final point for consideration before proceeding to the leitmotifs of LTP biochemistry, it is important to keep in mind the physiologic context of the biochemical changes we will be discussing. LTP is a complex cell biological phenomenon that impinges on many measurable physiologic, homeostatic and structural processes in the neuron. The single aspect of LTP that has received by far the most attention is the LTP-associated increase of synaptic strength, which results from either increasing neurotransmitter release or augmenting glutamate receptor function. A second physiologic effect in LTP is increased postsynaptic neuron excitability. This component of LTP is manifest as an increased likelihood of firing an action potential for a given synaptic current (Gribkov and Ashe 1984; Reymann et al. 1985; Madison and Nicoll 1986; Reymann et al. 1988; Chavez-Noriega et al. 1990; Heginbotham and Dunwiddie 1991; Chavez-Noriega and Stevens 1992; Wathey et al. 1992; Pockett et al. 1993). The phenomenon was first described as a high-frequency stimulation-induced increase in population spike (S) amplitude independent of an increased excitatory postsynaptic potential (EPSP) (E), resulting in the term E–S potentiation (Bliss and Lomo 1973). At present it is not entirely clear if the increased excitability is due to an intrinsic change in the postsynaptic neuron or an alteration in the excitation/inhibition ratio of the local hippocampal circuit, i.e. an alteration in GABAergic inhibition by local feed-forward interneurons. Future biochemical analysis of this component of LTP can only improve our understanding of the underlying mechanisms for E–S potentiation. Overall for this review we will focus our attention on biochemical signals resulting in altered physiologic, as opposed to structural or homeostatic, properties. Thus we will limit our discussion to biochemical mechanisms that ultimately result in altered glutamate receptor function, altered membrane biophysical properties, or altered neurotransmitter release.

**Leitmotifs in the biochemistry of LTP**

Having reviewed the established themes in the biochemistry and physiology of LTP (persistent biochemical signals; persistent kinase activity; molecules that modulate LTP vs those that are mandatory), and having defined our terms (E- and L-LTP; LTP induction and maintenance), we can posit the themes on which this review is focused. These themes are listed in Table 1 and the remainder of this text is organized accordingly. Each section will start by defining a particular theme from the vantage point of its physiologic correlate in LTP, followed by a discussion of the biochemical mechanisms underlying this physiology and, lastly, where appropriate, point out future avenues of investigation.

**Theme 1 — signal amplification**

LTP, assessed physiologically, exhibits a threshold stimulus intensity for its induction. That is, its induction appears to be triggered by a unitary event. Achieving a sufficient level of postsynaptic depolarization for opening NMDA channels certainly is a basis for this attribute; however, the postsynaptic biochemical events triggered by the resulting influx of Ca$^{2+}$ also likely contribute to this effect. Highly amplified biochemical systems tend to create a step function...
for triggering an effect – in this case LTP. The extent of signal amplification in the signal transduction cascades subserving the induction of LTP is quite striking and below we highlight six notable examples from the literature that exemplify feed-forward, feed-back and cross-talk amplification (Table 2).

**Phosphatase inhibition in LTP induction – inhibitor 1**
Phosphatase inhibition in a *feed-forward* manner serves to amplify kinase activation with LTP-inducing stimulation. The cAMP cascade is activated with LTP-inducing physiologic stimulation, and Blitzer et al. (1995, 1998) observed that activation of the cAMP pathway is necessary for E-LTP due at least in part to PKA-dependent activation of protein inhibitor 1 phosphatase. This mechanism amplifies autophosphorylation of CaMKII at Thr286 and the resultant Ca$^{2+}$-independent persistent CaMKII activity. Thus, Blitzer et al. (1995, 1998) concluded that inhibitor 1-dependent protein phosphatase inhibition via the cAMP pathway serves to gate CaMKII signaling during LTP induction. Similarly, Winder et al. (1998) have proposed that PKA activity relieves a calcineurin constraint on the induction of LTP. Based on their studies they suggest the existence of a novel, intermediate phase of LTP (I-LTP) that differs from E-LTP by requiring multiple trains for induction and requiring PKA activity. It differs from L-LTP by not requiring new protein synthesis. Phosphatase inhibition also is a likely site whereby activation of β-adrenergic receptors (β-AR) or dopamine receptors (DAR) can modulate LTP induction through phosphatase inhibition turning a subthreshold stimulus into one adequate for LTP induction (Otmakhova and Lisman 1996; Thomas et al. 1996). This is a very active area of contemporary investigation, utilizing a variety of transgenic animal technologies and specific phosphatase inhibitors.

**PKA amplification of CaMKII – neurogranin**
Phosphorylation-dependent regulation of the calpainin protein family member neurogranin is another example of a feed-forward amplification mechanism. A number of studies have identified the postsynaptic calmodulin binding protein neurogranin as a PKC substrate altered in LTP (reviewed in Gerendasy and Sutcliffe 1997) and this PKC target is particularly notable as it represents a potential site of interaction between the PKC and CaMKII systems in LTP. Neurogranin serves as a site of signal amplification by virtue of the fact that PKC phosphorylation of neurogranin leads to a decrease in its affinity for calmodulin, leading to increased free calmodulin levels postsynaptically and an augmentation of calmodulin-dependent processes in LTP induction. In fact, recent studies by Huang and co-workers have initiated the testing of this hypothesis using a neurogranin-deficient mouse line (Pak et al. 1999).

**Erk MAPK**
The erk MAPK cascade plays a critical role in multiple forms of LTP, and in several types of learning in animals. The erk MAPK cascade is the prototype highly amplified signal transduction cascade, as it is a serial linkage of enzymatic catalytic events. Regulation of the erk MAPK cascade is complex (reviewed in Cobb and Goldsmith 1995), yet one salient feature of the cascade is that erk MAPK activity is exclusively regulated by MEK, an upstream dual-specificity kinase that phosphorylates erk MAPK on both a Tyr and a Thr residue. This dual phosphorylation is both necessary and sufficient for erk MAPK activation. MEK in turn is regulated by either of two homologous upstream kinases, Raf-1 or B-raf, themselves subject to amplified regulation by various receptor–effector coupling mechanisms. Thus, the amplification achieved by the serial linkage of three protein kinases is potentially tremendous.

The specific mechanisms operating to regulate erk MAPK activation in LTP are not yet entirely worked out. Recent investigations have determined that PKC activation leads to activation of erk MAPK in hippocampal area CA1 and the cAMP cascade also activates erk MAPK in area CA1 (English and Sweatt 1996a,b; Impey et al. 1998; Roberson et al. 1999). Therefore, we hypothesize that these two kinases regulate erk MAPK activation in LTP biochemistry, although many other upstream regulatory mechanisms are currently under investigation by this and other laboratories. The observation that both PKA and PKC can regulate erk MAPK activity has an interesting but as yet untested implication; the possibility of synergistic activation of erk MAPK by conjoint low-level activation of PKA and PKC.

**Amplification through augmented membrane depolarization – Kv4.2**
The Shal-type K$^+$ channel Kv4.2 is a voltage-dependent K$^+$ channel localized to the dendrites of hippocampal pyramidal neurons. Its localization, physiologic properties, and known regulation by protein kinases make it an ideal candidate as a
positive feedback amplifier of neuronal excitability in LTP. In recent studies we have observed that PKC, PKA, CaMKII and erk MAPK phosphorylate Kv4.2, although to date it is unknown whether the phosphorylation of Kv4.2 by these kinases is altered in LTP. Hoffman et al. (1997) found that in the hippocampus, voltage-dependent, transient K⁺ channels of the Shal superfamily (Kv4 channels) regulate both the excitability of pyramidal neurons and the magnitude of the EPSP produced in response to synaptic activity. Moreover, Hoffman and Johnston (1998) recently showed that voltage-dependent activation of these currents in the dendrites of CA1 pyramidal neurons is decreased by either PKC or PKA. Therefore, modulation of the properties of these channels (e.g. voltage-dependence, inactivation rate, number of channels, distribution) is an appealing potential site for regulation of pyramidal neuron excitability in LTP (e.g. E–S potentiation; see Functional Integration). Protein kinase phosphorylation of this channel would serve as a signal amplification mechanism because decreasing the function of this channel promotes membrane depolarization and enhances Ca²⁺ influx via any membrane potential-dependent mechanism such as activation of voltage-gated Ca²⁺ channels or NMDA receptor function. The Johnston, Winder and O’Dell laboratories are actively pursuing this line of investigation at present.

Positive feedback of receptor activation – AMPA and NMDA receptors

LTP in area CA1 results in increased synaptic strength through increased glutamate receptor responsiveness due, in part, to an underlying increase in α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor unitary conductance (Benke et al. 1998; also see Derkach et al. 1999). AMPA receptor-mediated current is potentiated during LTP by CaMKII phosphorylation (Barria et al. 1997b). AMPA receptor-mediated synaptic currents are similarly potentiated when PKC catalytic subunit is intracellularly perfused in cultured CA1 hippocampal neurons (Wang et al. 1994). In vitro, it has been demonstrated that both PKC and CaMKII can phosphorylate Ser831 on the carboxyl-terminal tail of the GluR1 subunit (Roche et al. 1996; Barria et al. 1997a; Mammen et al. 1997). These phosphorylation events are one basis of E-LTP expression: potentiated synaptic AMPA receptor responses. However, these same mechanisms may serve to amplify postsynaptic membrane depolarization during LTP induction.

PKA phosphorylation of AMPA receptors can also occur on the carboxyl-terminal tail of the GluR1 subunit at Ser485; a site unique from the PKC/CaMKII site (Roche et al. 1996). The exact role of PKA phosphorylation of AMPA receptors in synaptic function and potentiation is unclear. PKA phosphorylation is required for maintaining basal AMPA receptor function (Knapp and Dowling 1987; Wang et al. 1991) and has been shown to potentiate AMPA receptor current in some preparations (Greengard et al. 1991; Roche et al. 1996) but not in others (Blitzer et al. 1995; Kameyama et al. 1998; Rosenmund et al. 1994). One interpretation of the data is that synaptic AMPA receptors in the hippocampus are highly basally phosphorylated by PKA (see Lee et al. 1998) and one consequence of PKA activation during LTP induction may be saturation of AMPA receptor PKA phosphorylation sites during the time course of PKC and CaMKII activation. Since PKA activity is necessary for basal AMPA receptor function, PKA phosphorylation of AMPA receptors during LTP induction may amplify the potentiating effect of PKC and CaMKII phosphorylation. This is an area of inquiry currently under investigation by the laboratories of Huganir, Soderling and colleagues.

Modulation of NMDA receptors by the non-receptor protein tyrosine kinase Src is emerging as another example for the positive feedback amplification leitmotif. Initial studies in this area indicated that protein tyrosine phosphorylation is necessary for the induction of LTP based on effects of broad-spectrum protein kinase inhibitors (O’Dell et al. 1991). In one of the first studies of a genetically modified animal, deficits in LTP were detected in mice lacking the src family member fyn (Grant et al. 1992). Moreover, activity of the tyrosine kinase Src is increased with LTP-inducing stimuli in CA1 pyramidal neurons and Src activity is necessary for LTP induction (Lu et al. 1998; Huang and Hsu 1999). Src, in a positive feedback manner, regulates the function of NMDA receptor currents, which are potentiated with NMDA receptor-dependent LTP in CA1 (Muller and Lynch 1990; Wang and Salter 1994; Yu et al. 1997). Potentiated NMDA receptor currents lead to increased Ca²⁺ influx and likely boost downstream Ca²⁺ dependent signaling cascades. The activation of Src appears to be dependent on PKC activity; Src activation can be achieved indirectly via PKC activation through G protein-coupled receptors (Lu et al. 1999). While protein tyrosine phosphorylation clearly is necessary and sufficient for NMDA receptor-dependent LTP in CA1, at present we do not know much about the upstream regulators/modulators of Src activity. Therefore at present it is difficult to state precisely how Src fits into biochemical models of LTP induction, including our own.

Positive feedback of the Ca²⁺ signal – Ca²⁺ channels

Another example of feedback amplification is the Ca²⁺ induced potentiation of Ca²⁺ channel function with LTP. NMDA receptor activation during LTP induction leads to increases in intracellular Ca²⁺ and cAMP levels, as well as activation of PKA (Chetkovich et al. 1991; Roberson and Sweatt 1996). NMDA receptor activation and increased cAMP levels lead to increased Ca²⁺ channel fractional open time (Chetkovich et al. 1991), presumably through Ca²⁺

channel phosphorylation by PKA (Gray and Johnston 1987; Hell et al. 1995). These phenomena potentially set up an amplification loop for Ca\(^{2+}\) influx in area CA1 during LTP induction: synaptic stimulation activates NMDA receptors allowing Ca\(^{2+}\) influx that leads to an enhancement of further Ca\(^{2+}\) influx via increased cAMP (and PKA activity) and subsequent increased voltage-gated Ca\(^{2+}\) channel activity.

Release of Ca\(^{2+}\) from intracellular stores during LTP induction is another example of feed-back amplification. Two regulatory mechanisms for such release have been described: release triggered by inositol 1,4,5-triphosphate (IP3), a second messenger generated downstream of many metabotropic receptors (IP3-induced release) and release stimulated by influx of extracellular Ca\(^{2+}\) (Ca\(^{2+}\)-induced Ca\(^{2+}\) release). Thapsigargin, which depletes intracellular Ca\(^{2+}\) stores and thus interferes with both mechanisms, and dantrolene, which antagonizes the ryanodine receptor and inhibits Ca\(^{2+}\)-induced Ca\(^{2+}\) release, both block LTP induction (Obenaus et al. 1989; Harvey and Collingridge 1992).

In summary, there is extensive feed-forward and feed-back amplification among the signal transduction mechanisms operating in LTP induction and expression, serving to boost the LTP-inducing Ca\(^{2+}\) signal and to promote a sharp threshold for LTP induction. Unfortunately for the experimentalist, an additional implication of the extensive cross-talk effects of one signal transduction mechanism on another in LTP is that it is effectively impossible to selectively inhibit any one enzyme involved in LTP induction. Even an absolutely selective inhibitor (or genetic manipulation) is likely to have pronounced secondary consequences on the other signal transduction molecules involved in LTP induction.

A caveat: negative feed-back
Finally, another aspect of modulation of LTP induction that will not be discussed in this section, but nonetheless should be kept in mind, is negative feed-back signaling cascades. For example, NMDA receptor activation leads to increased intracellular Ca\(^{2+}\) concentration and NO production that subsequently can inhibit NMDA receptor function and block the expression of LTP by subsequent LTP-inducing stimuli (Izumi et al. 1992). A similar caveat concerns competitive signaling cascades such as concomitant activation of a phosphatase and a kinase by Ca\(^{2+}\) and calmodulin: the antagonistic enzymes, calcineurin and CaMKII, are an example (Hashimoto et al. 1988).

Theme 2 – signal integration
A second theme to emerge from studies of LTP induction and expression is integration of biochemical signals in the temporal, functional and spatial domains. As each of these categories has unique physiologic attributes, we discuss them separately below. The net effect of signal integration mechanisms such as those described below is to allow disparate physiologic stimuli to achieve additive, synergistic, or redundant effects in the induction or expression of LTP.

2A – temporal integration
It is known that repetitive tetanization can elicit uniquely long-lasting physiologic effects. In this context it is important to note that the biochemical processes that we have been discussing are not terminated instantaneously, but typically decay with a half-life on the order of minutes. Thus, delivery of one stimulus may serve to amplify subsequent temporally spaced stimuli, allowing for temporal integration of signals. This is possibly the means by which multiple, spaced tetanic stimuli are able to selectively produce unique effects. A striking example of temporal integration at the level of a single molecule, CaMKII, has been published by De Koninck and Schulman (1998). In this enlightening paper the frequency-dependence of CaMKII autonomous activity was demonstrated in vitro. The rise-time of Ca\(^{2+}\)/calmodulin-induced CaMKII activity was dependent on the frequency and duration of pulses of activating solution (Ca\(^{2+}\), calmodulin, ATP). In addition, acquisition of CaMKII autonomy was temporally dependent on calmodulin concentration (Fig. 1). Therefore, intracellular Ca\(^{2+}\) spike variations (and subsequent calmodulin availability) are integrated over time by CaMKII, with the readout being autophosphorylation at Thr286 and second-messenger-independent kinase activity.

2B – functional integration
AMPA receptors and Kv4.2
As mentioned above, there are two general physiologic outputs evident in E-LTP expression. The first is an increase in synaptic strength, that is, an increase in the EPSP that results from increased levels of glutamate receptor activation. As described above, one mechanism contributing to this phenomenon is fairly well understood – phosphorylation of AMPA receptors. The level of AMPA receptor phosphorylation increases in E-LTP at a site that can be phosphorylated by either CaMKII or PKC, and increased phosphorylation at this site results in increased receptor current (Barria et al. 1997b). As both PKC and CaMKII are persistently activated in E-LTP, a parsimonious explanation for the increased synaptic strength is increased AMPA receptor phosphorylation by one or both of these kinases. In this context AMPA receptors may be serving to integrate signal from two independent sources: CaMKII and PKC (Fig. 2).

The second type of physiologic change in LTP is E–S potentiation. While the mechanisms underlying this LTP-associated increase in neuronal excitability are unknown (however, see Lu et al. 2000), recent studies such as those
described above indicate that phosphorylation and functional down-regulation of the K\textsuperscript{+} channel Kv4.2 is a feasible possibility. As already mentioned, we have evidence that Kv4.2 is a substrate for all four kinases known to be involved in LTP: PKC, PKA, CaMKII, and erk MAPK. Thus, both AMPA receptors and Kv4.2 channels can serve as functional integrators of protein kinase activity during LTP induction, by serving as convergence points for their actions (Fig. 2).

**Erk MAPK**

There is a plethora of evidence that erk MAPK serves in hippocampal area CA1 as an integrator of signals from a wide range of cell surface receptors. For example, activation of both the cAMP cascade and the PKC cascade leads to secondary activation of erk MAPK in hippocampal area CA1 (Roberson et al. 1999). In addition, hippocampal erk MAPK is activated by β-AR, metabotropic glutamate, muscarinic acetylcholine and DAR activation (Roberson et al. 1999). Moreover, brain-derived neurotrophic factor (BDNF) also elicits erk MAPK activation in this region (Gottschalk et al. 1999). An important implication of these data is that these receptor systems may utilize the erk MAPK cascade as a signal integrator to modulate LTP induction in area CA1. Consistent with this idea, Winder et al. (1999) have recently observed that the β-AR modulation of LTP induction is blocked by MEK inhibitors. This prospect is particularly interesting because erk MAPK appears to serve a privileged role as a regulator of gene expression via regulation of Ca\textsuperscript{2+}/cAMP response element binding (CREB) protein phosphorylation (Impey et al. 1998.; Roberson et al. 1999).

Thus, these studies coupled with prior studies indicating that both the PKA and PKC pathways can elicit hippocampal erk MAPK activation, suggest that erk MAPK serves as an integrator of a wide variety of convergent receptor-generated messages, allowing for the functional integration of diverse cell surface signals. This is likely to be an increasingly active area for future investigation.

**2C — spatial integration**

**NMDA receptor**

As has been widely discussed in the literature, the NMDA receptor integrates an electrical signal (membrane...
depolarization) and a chemical signal (synaptic glutamate). These two signals may originate in spatially widely separated loci, as illustrated by 'pairing' LTP induced by coincident action potential generation and synaptic stimulation (Fig. 3). Thus, in this context the NMDA receptor is serving not only as a coincidence detector but also as an integrator of information arising from spatially distinct sources.

Erk MAPK

As described above, erk MAPK is activated in area CA1 by NMDA receptors, BDNF receptors, β-AR, DAR, muscarinic acetylcholine receptors, and metabotropic glutamate receptors. The diversity of erk MAPK regulation in the hippocampus suggests the possibility of a role for the erk MAPK cascade in integrating signals from a wide variety of cellular

Fig. 3 Spatial integration. NMDA receptors integrate membrane depolarization and chemical transmission, in this example LTP induced by 'pairing' coincident action potential generation and synaptic stimulation. The electrical signal being generated remotely from the site of synaptic transmission, erk MAPK integrates dendritic activity that potentially results in changes in gene transcription through CREB phosphorylation.

Fig. 4 Signal transduction cascades operating in LTP. This schematic represents a presynaptic glutamate release site and a postsynaptic dendritic spine with neurotransmitter receptors, voltage-dependent channels and signal transduction machinery. (CaM) calmodulin; (NOS) NO synthase; (AC) adenylyl cyclase; (*PKC) autophosphorylated (persistently activated) PKC or the autonomously active proteolytic form of PKC (ζ isoform); (*CaMKII) CaMKII, either transiently or persistently activated; (P-I) protein phosphatase inhibitor 1; (PP) protein phosphatase; (VGCC) voltage-gated calcium channels; (Kv) voltage-dependent K+ channels; (GLU) glutamate.
compartments. Moreover, as erk MAPK is a critical regulator of CREB phosphorylation in area CA1, it has the capacity to integrate a wide variety of synaptic signals at the level of the nucleus – the genome-containing compartment (Fig. 3). While this idea is speculation at present, recent findings from the Storm, Sweatt and Tsien labs has suggested that spatially compartmentalized signals may in fact selectively activate erk MAPK in hippocampal neurons.

Theme 3 – signal coordination

LTP has a problem to solve. It is a heterogenous physiologic and biochemical response to a discrete triggering event. How are the multiple downstream effects of LTP induction achieved in a coordinated fashion? One solution to this problem is through the pluriptotent nature of protein kinases, such that activation of a single protein kinase typically elicits changes in a wide variety of downstream effectors (Fig. 4). Tracing the biochemical consequences of activation of a single class of protein kinase activated during LTP induction illustrates this point. For example, PKC (and CaMKII) can phosphorylate AMPA receptors and increase current conductance (Derkach et al. 1999) resulting in enhanced glutamatergic synaptic transmission. PKC phosphorylation of $K^+$ channels can increase neuronal excitability by down-regulating $K^+$ currents through shifting the voltage dependence of their activation (Hoffman and Johnston 1998). Via the erk MAPK cascade, PKC activation can lead to phosphorylation of CREB, initiating changes in gene transcription (Roberson et al. 1999). Although activation of PKC during LTP impacts a variety of molecular targets, each of these effectors is harmonized towards expressing the same neuronal phenotype – LTP. Similar examples can be drawn for the PKA and CaMKII cascades.

Conclusion

Activation of multiple kinase pathways during LTP induction results in well-coordinated biochemical signals that increase neuronal excitability and lay the groundwork for potentiated synaptic transmission. Through synergistic multicomponent signaling, the activities of PKA, CaMKII and erk MAPK generate the establishment of LTP. PKA phosphorylation of AMPA receptors potentiates their currents, possibly through an influence on peak open probability (Roche et al. 1996; Banke et al. 2000). PKA activity can also increase neuronal excitability by phosphorylating $K^+$ channels (Hoffman and Johnston 1998) and $Ca^{2+}$ channels (Gray and Johnston 1987; Hell et al. 1995). Phosphorylation of inhibitor-1 by PKA decreases protein phosphatase 1 activity which then positively modulates the CaMKII cascade (Blitzer et al. 1998). Similarly, CaMKII (and PKC) activity can: increase glutamatergic synaptic transmission by phosphorylating AMPA receptors (Derkach et al. 1999; Barria et al. 1997b), regulate synaptic vesicle release by phosphorylating synaptic vesicle proteins (Fukunaga et al. 1995; Nayak et al. 1996), modify synaptic structure by phosphorylating cytoskeleton proteins (Fukunaga et al. 1995) and positively feed-back onto the protein kinase cascades by phosphorylating and inhibiting protein phosphatase 2a (Fukunaga et al. 2000). Multiple downstream targets of erk MAPK include ion channels, cytoskeletal proteins and transcription factors (Bailey et al. 1997; Impey et al. 1998; Roberson et al. 1999). At first glance this biochemical complexity appears overwhelming and a daunting computational task for the neuron. Yet from inspection of each protein kinase cascade, emerges the theme that activation of single signaling cascades produces multicomponent but coordinated molecular responses that are geared toward a unified cellular outcome: a persistent enhancement of synaptic transmission.

Figure 4 presents a model for LTP diagramming a few of the specific molecular examples we have pointed out in this short overview of the biochemistry of LTP induction. It is important to note that neither the model nor this review are comprehensive. Many different types of signal transduction cascades and ion channels are potentially involved in LTP, and each of these molecular species may perform critical functions in this complex cellular phenomenon. Nevertheless, our hope is that by highlighting a few specific themes emerging from studies of LTP biochemistry, we have provided a useful categorization into which new discoveries can be synthesized.

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